Fig. 1

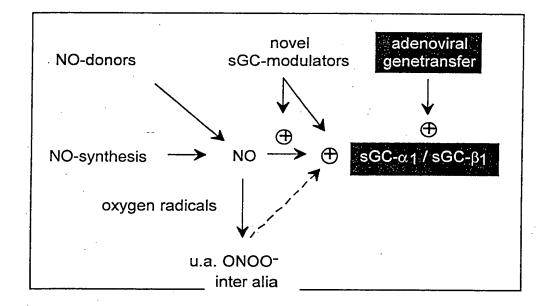
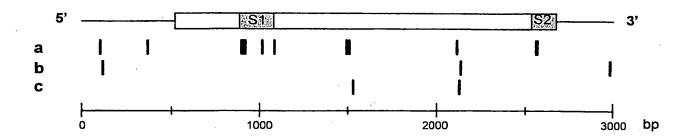


Figure 2

N				c	$sGC\alpha_1$ (rat, bovine)
	regulatory domain	sGC homology- domain	Cyclase- domain		
N				C	"sGCα₃" (human)
_	S1			S2	·



a: nucleotide insertions

C95, C367, T891, G900, T903, G913, T1006, G1074, G1487, A1488, A1489, G2108, G2555, T2560

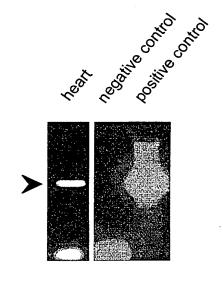
b: nucleotide delentions

T between G111 and T112, T between T2128 and G2129, T between G2975 and T2976

c: nucleotide exchanges C1525>G, G2125>A

Α

PCR determination of $hsGC\alpha1$



В

PCR determination of hsGCβ1

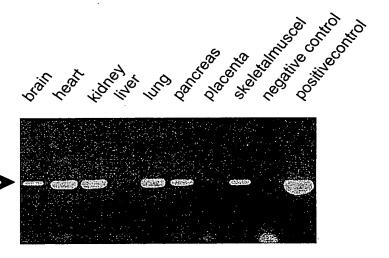
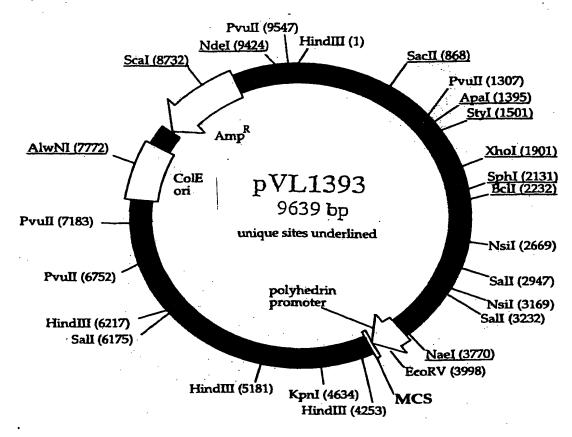


Figure 5
pVL1393 Baculovirus Transfer Vector



multiple cloning site (MCS) of pVL1393 with the unique restriction sites

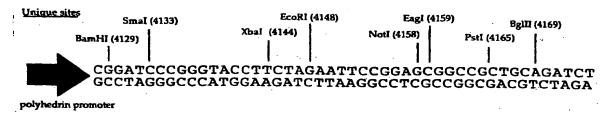
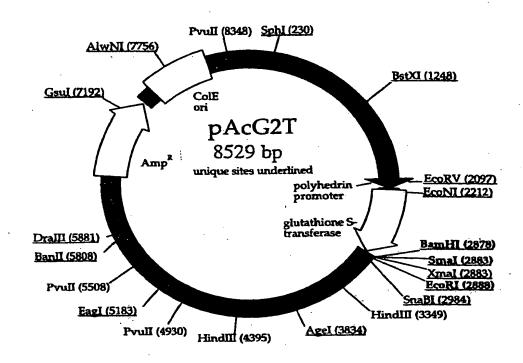


Figure 6
pAcG2T Baculovirus Transfer Vector



multiple cloning site (MCS) of pAcG2T downstream of glutathione-S-transferase sequence (GST) with the thrombin cleavage site and the unique restriction sites

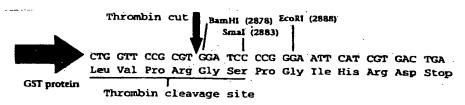
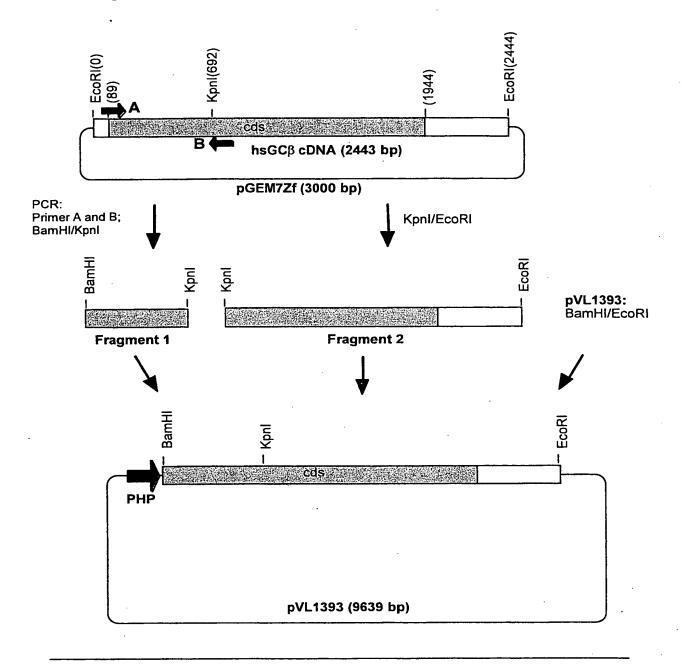
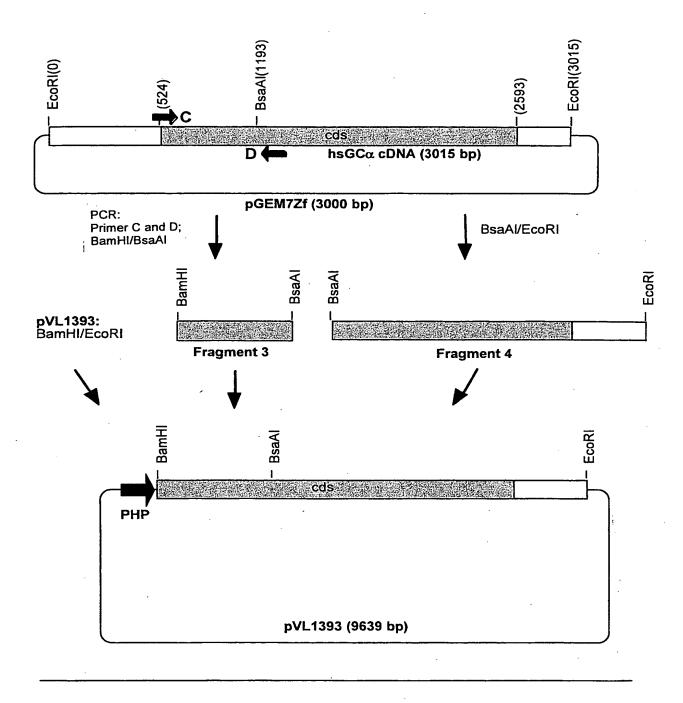


Figure 7: Cloning of hsGCβ in pVL1393



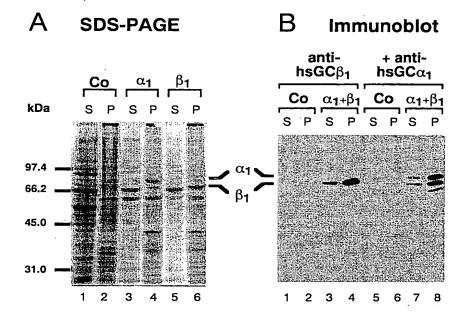
- Primer: 5' AAAA GGATCC ATGTACGGATTTGTGAAT 3' (116)BamHI (89)
 - В 3' CCATGG GTCCTTAGTGCGTA 5' (692) Kpnl (711)

Figure 8: Cloning of hsGC α in pVL1393



- Primer: C 5' AAAA GGATCC ATGTTCTGCACGAAGCTC 3'
 BamHI (524) (541)
 - **D** 3' GGAGGGACGAAGGTATTA 5' (1232) (1249)

Figure 9



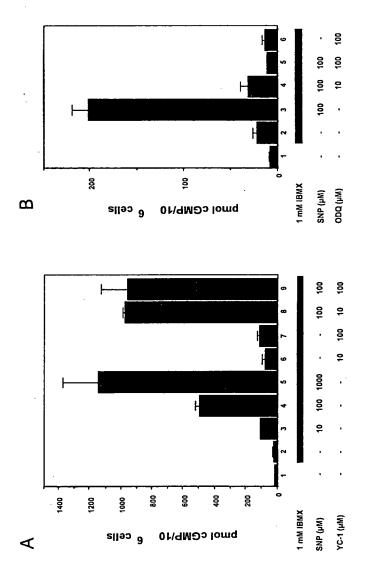


Fig. 11

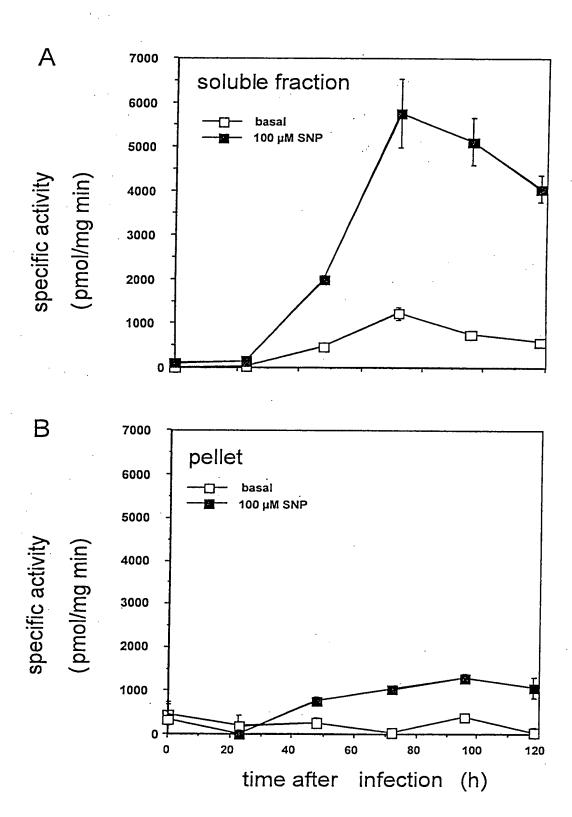
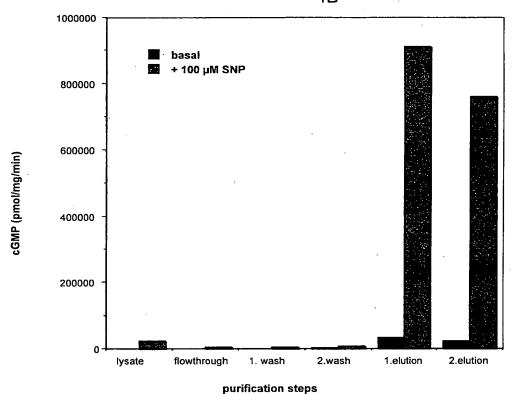


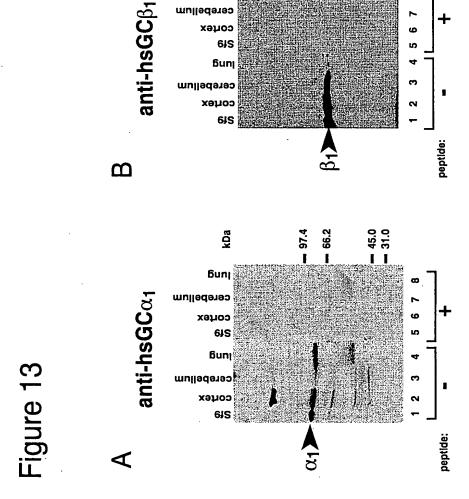
Figure 12: Purification of GST-hsGCalpha1/beta1 on GSH-Sepharose 4B



1 97.4

- 66.2

– 45.0 **–** 31.0



кDа

6un_l

cerebellum couex 612

Figure 14

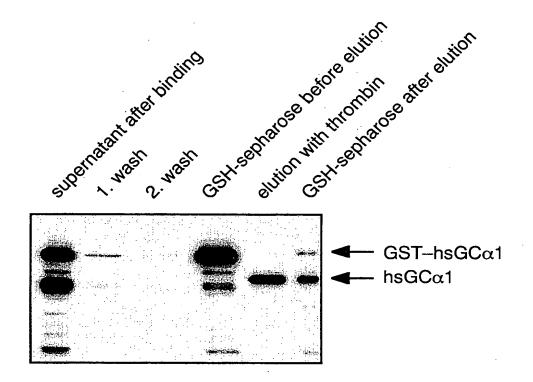


Figure 15: Purification of hsGC $\alpha 1/\beta 1$ in a Coomassie stained SDS polyacryamide gel

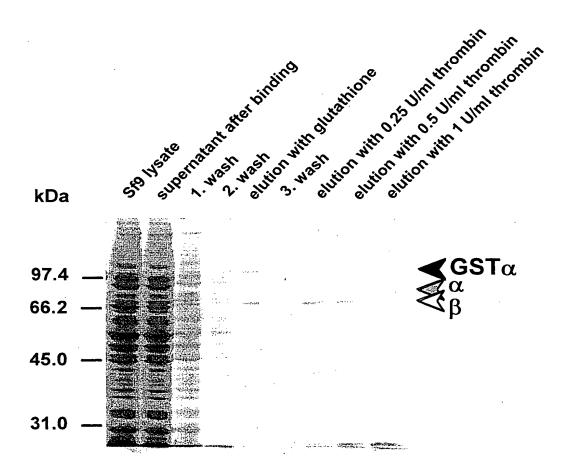


Figure 16: Construction of the hsGC-adenovectors

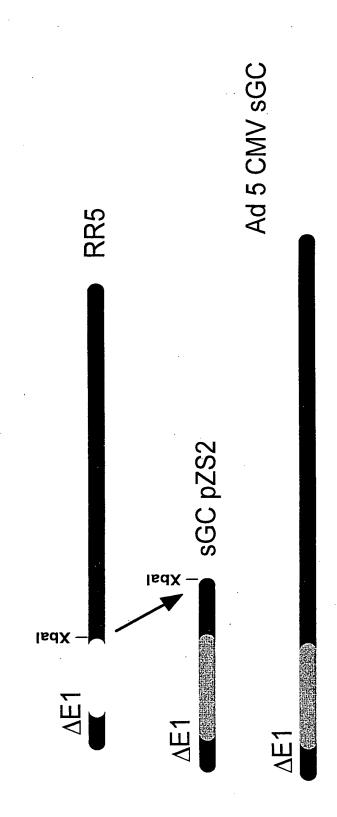
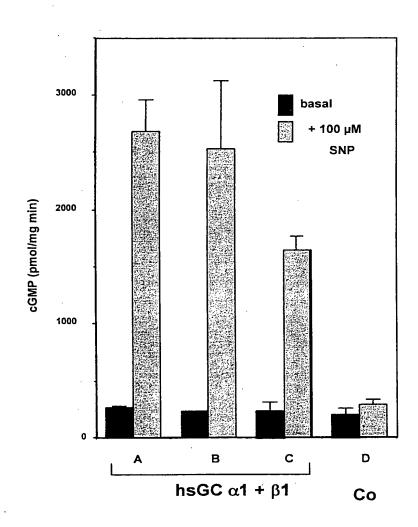


Figure 17: Expression of human sGC in adenovirus-infected EA.hy926 cells



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TTCCTACACT		TAGAGCAGCG			
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ATGTGCGGAT	TTGCGAGGCG	CGCCCTGGAG	CTGCTAGAGA	TCCGGAAGCA	CAGCCCCGAG
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Ser Arg Ser Arg Val Tyr Leu His Thr Leu Ala Glu Ser Ile Cys Lys
Leu Ile Phe Pro Glu Phe Glu Arg Leu Asn Val Ala Leu Gln Arg Thr
Leu Ala Lys His Lys Ile Lys Glu Ser Arg Lys Ser Leu Glu Arg Glu
Asp Phe Glu Lys Thr Ile Ala Glu Gln Ala Val Ala Ala Gly Val Pro
Val Glu Val Ile Lys Glu Ser Leu Gly Glu Val Phe Lys Ile Cys
Tyr Glu Glu Asp Glu Asn Ile Leu Gly Val Val Gly Gly Thr Leu Lys
Asp Phe Leu Asn Ser Phe Ser Thr Leu Leu Lys Gln Ser Ser His Cys
Gln Glu Ala Gly Lys Arg Gly Arg Leu Glu Asp Ala Ser Ile Leu Cys
Leu Asp Lys Glu Asp Asp Phe Leu His Val Tyr Tyr Phe Phe Pro Lys
Arg Thr Thr Ser Leu Ile Leu Pro Gly Ile Ile Lys Ala Ala Ala His
Val Leu Tyr Glu Thr Glu Val Glu Val Ser Leu Met Pro Pro Cys Phe
His Asn Asp Cys Ser Glu Phe Val Asn Gln Pro Tyr Leu Leu Tyr Ser
Val His Met Lys Ser Thr Lys Pro Ser Leu Ser Pro Ser Lys Pro Gln
Ser Ser Leu Val Ile Pro Thr Ser Leu Phe Cys Lys Thr Phe Pro Phe
His Phe Met Phe Asp Lys Asp Met Thr Ile Leu Gln Phe Gly Asn Gly
Ile Arg Arg Leu Met Asn Arg Arg Asp Phe Gln Gly Lys Pro Asn Phe
Glu Glu Tyr Phe Glu Ile Leu Thr Pro Lys Ile Asn Gln Thr Phe Ser
Gly Ile Met Thr Met Leu Asn Met Gln Phe Val Val Arg Val Arg Arg
Trp Asp Asn Ser Val Lys Lys Ser Ser Arg Val Met Asp Leu Lys Gly
Gln Met Ile Tyr Ile Val Glu Ser Ser Ala Ile Leu Phe Leu Gly Ser
Pro Cys Val Asp Arg Leu Glu Asp Phe Thr Gly Arg Gly Leu Tyr Leu
Ser Asp Ile Pro Ile His Asn Ala Leu Arg Asp Val Val Leu Ile Gly
Glu Gln Ala Arg Ala Gln Asp Gly Leu Lys Lys Arg Leu Gly Lys Leu
Lys Ala Thr Leu Glu Gln Ala His Gln Ala Leu Glu Glu Glu Lys Lys
Lys Thr Val Asp Leu Cys Ser Ile Phe Pro Cys Glu Val Ala Gln
Gln Leu Trp Gln Gly Gln Val Val Gln Ala Lys Lys Phe Ser Asn Val
Thr Met Leu Phe Ser Asp Ile Val Gly Phe Thr Ala Ile Cys Ser Gln
Cys Ser Pro Leu Gln Val Ile Thr Met Leu Asn Ala Leu Tyr Thr Arg
Phe Asp Gln Gln Cys Gly Glu Leu Asp Val Tyr Lys Val Glu Thr Ile
Gly Asp Ala Tyr Cys Val Ala Gly Gly Leu His Lys Glu Ser Asp Thr
His Ala Val Gln Ile Ala Leu Met Ala Leu Lys Met Met Glu Leu Ser
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Ile Asp End
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Glu Ile Leu Gln Met Phe Gly Lys Met Phe Phe Val Phe Cys Gln Glu
Ser Gly Tyr Asp Thr Ile Leu Arg Val Leu Gly Ser Asn Val Arg Glu
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Pro Gly Met Arg Ala Pro Ser Phe Arg Cys Thr Asp Ala Glu Lys Gly
Lys Gly Leu Ile Leu His Tyr Tyr Ser Glu Arg Glu Gly Leu Gln Asp
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Lys Gly Lys Lys Glu Pro Met Gln Val Trp Phe Leu Ser Arg Lys Asn
Thr Gly Thr Glu Glu Thr Lys Gln Asp Asp end
```

22/28

Figure 22

Phe Thr Pro Arg Ser Arg Glu Glu Leu Pro Pro Asn Phe Pro

Figure 23

Lys Gly Lys Lys Glu Pro Met Gln Val Trp Phe Leu Ser Arg Lys Asn Thr Gly Thr Glu Glu Thr

upper primer

AAAAGGATCC ATGTTCTGCA CGAAGCTC

lower primer

ATTATGGAAG CAGGGAGG

Figure 25

upper primer

AAAAGGAT.CC ATGTACGGAT TTGTGAAT

lower primer

ATGCGTGATT CCTGGGTACC

Figure 26

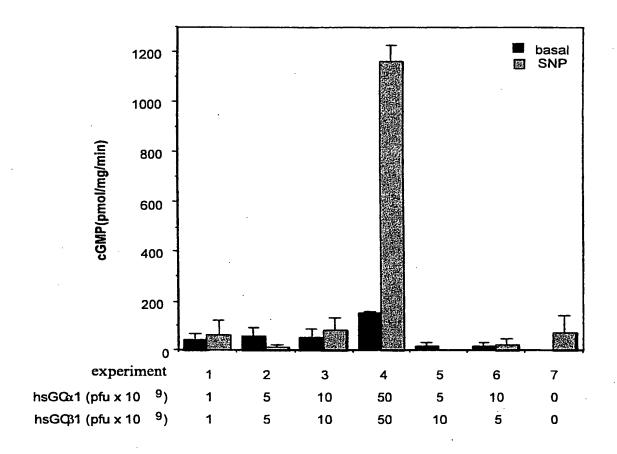


Figure 27

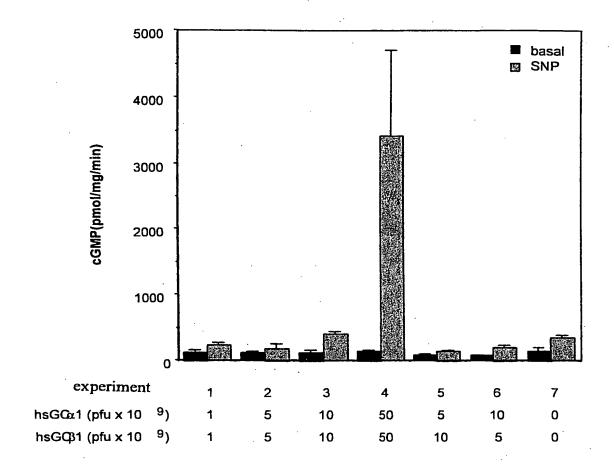


Figure 28

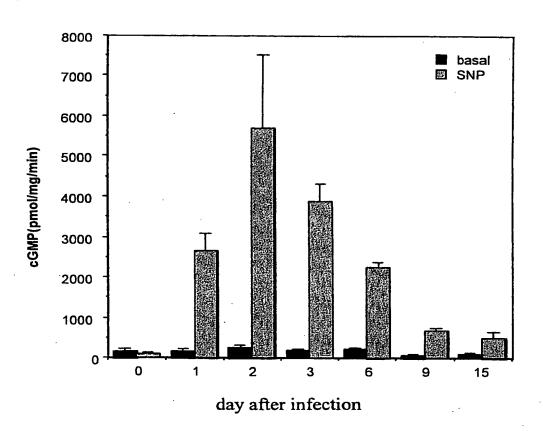


Figure 29

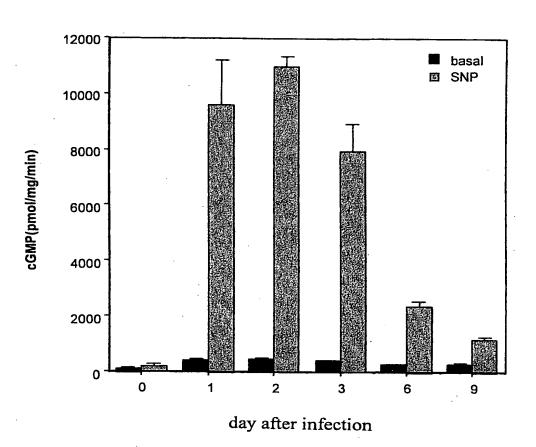


Figure 30

